Architecture of RNA Polymerase II

Structural Clues Help Unravel Intricacies of DNA Transcription

Before a cell can begin to divide or differentiate, the genetic information within the cell's DNA must be copied, or "transcribed," onto complementary strands of RNA. RNA polymerase II (pol II) is an enzyme that, by itself, can unwind the DNA double helix, synthesize RNA, and proofread the result. When combined with other molecules that regulate and control the transcription process, pol II is the key to successful interpretion of an organism's genetic code. However, the size, complexity, scarcity, and fragility of pol II complexes have made analysis of these macromolecules by x-ray crystallography a formidable challenge. A team of structural biologists has met this challenge using data obtained from both the Stanford Synchrotron Radiation Laboratory and the Macromolecular Crystallography Facility at the ALS. The resultant high-resolution model of a 10-subunit pol II complex suggests roles for each of the subunits and will allow researchers to begin unraveling the intricacies of DNA transcription and its role in gene expression.

In this work, the researchers studied the pol II enzyme from the yeast *Saccharomyces cerevisiae*, which is likely to be an excellent model for the human enzyme in light of its highly similar gene sequences. It is also the best-characterized form of the pol II enzyme, having been the subject of many biochemical and low-resolution structural studies in the past. To obtain a high-resolution structure, the research team drew on its considerable expertise in the preparation of protein crystals: two-dimensional crystals of pol II (minus two small subunits found to impede crystal growth) were used as seeds for growing three-dimensional crystals. These crystals, when produced in an inert atmosphere to prevent oxidation, enabled the collection of data to 3.5-angstrom resolution. The addition of a final soaking procedure to produce uniform crystals, combined with high-brightness x-ray sources, resulted in a resolution of 3.0 angstroms.

The current results bring into focus the somewhat fuzzy features previously observed in or inferred from earlier experiments. More importantly, the structural details suggest possible explanations for some of the unusual characteristics of this enzyme, which include a high processivity (the ability to synthesize very long strands of RNA) and the tendency to work in periodic spurts separated by pauses. While it is known that additional proteins (transcription factors) play a role in controlling the activity of pol II (for example, restarting after a pause), scientists have yet to understand how such proteins interact with pol II binding sites to perform their various functions. The pol II model reported here establishes the positions of the various subunits and provides detailed information about the DNA/RNA binding domains.

The data reveal two main subunits (Rpb1 and Rpb2) separated by a deep cleft where DNA can enter the complex. At the end of the cleft is the active site, where the DNA can be unwound for a short distance (the "transcription bubble") and a DNA/RNA hybrid can be produced. Two prominent grooves lead away from the active site, either of which could accommodate the exiting RNA transcript. An opening below the active site may allow the entry of nucleotides (for manufacturing RNA) and transcription factors (for regulating the process). The same opening may provide room for the leading end of the RNA strand during "backtracking" maneuvers, which are important for proofreading and for traversing obstacles such as DNA damage. Other notable features that might help account for the great stability of this transcribing complex include a pair of "jaws" that appear to grip the DNA strands as they enter the complex and, closer to the active site, a clamp on the DNA that could possibly be locked in the closed position by the presence of RNA.

The high-resolution pol II structure reported here is a landmark achievement, pulling together threads from numerous diverse research efforts into a cohesive whole. Further study should yield many new insights into the detailed mechanisms of pol II and its transcription factors. Construction of an atomic model is already well underway. ■

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P. Cramer, D.A. Bushnell, J. Fu, A.L. Gnatt, B. Maier-Davis, N.E. Thompson, R.R. Burgess, A.M. Edwards, P.R. David, R.D. Kornberg, "Architecture of RNA Polymerase II and Implications for the Transcription Mechanism," *Science* **288**, 640 (2000).

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Structural Clues Help Unravel Mystery of DNA Transcription

- **RNA polymerase II (pol II) enzyme**
  - *Unwinds DNA, synthesizes RNA, proofreads result*
  - *Operates with high processivity, interrupted by pauses*
  - *Is a large, complex, scarce, and fragile molecule: difficult to crystallize!*
- **Crystallographic success dependent on**
  - *Long history of ingenuity, expertise in growing crystals*
  - *Availability of high-brightness x-ray sources*
- **Suggestive features of high-resolution (3-Å) structure**
  - *Cleft for DNA entry, grooves for RNA exit*
  - *Opening for entry of nucleotides, transcription factors*
  - *Room for backtracking maneuvers*
  - *"Jaws" and "clamp" for stabilizing and locking DNA in place*
  - *Construction of atomic model underway*

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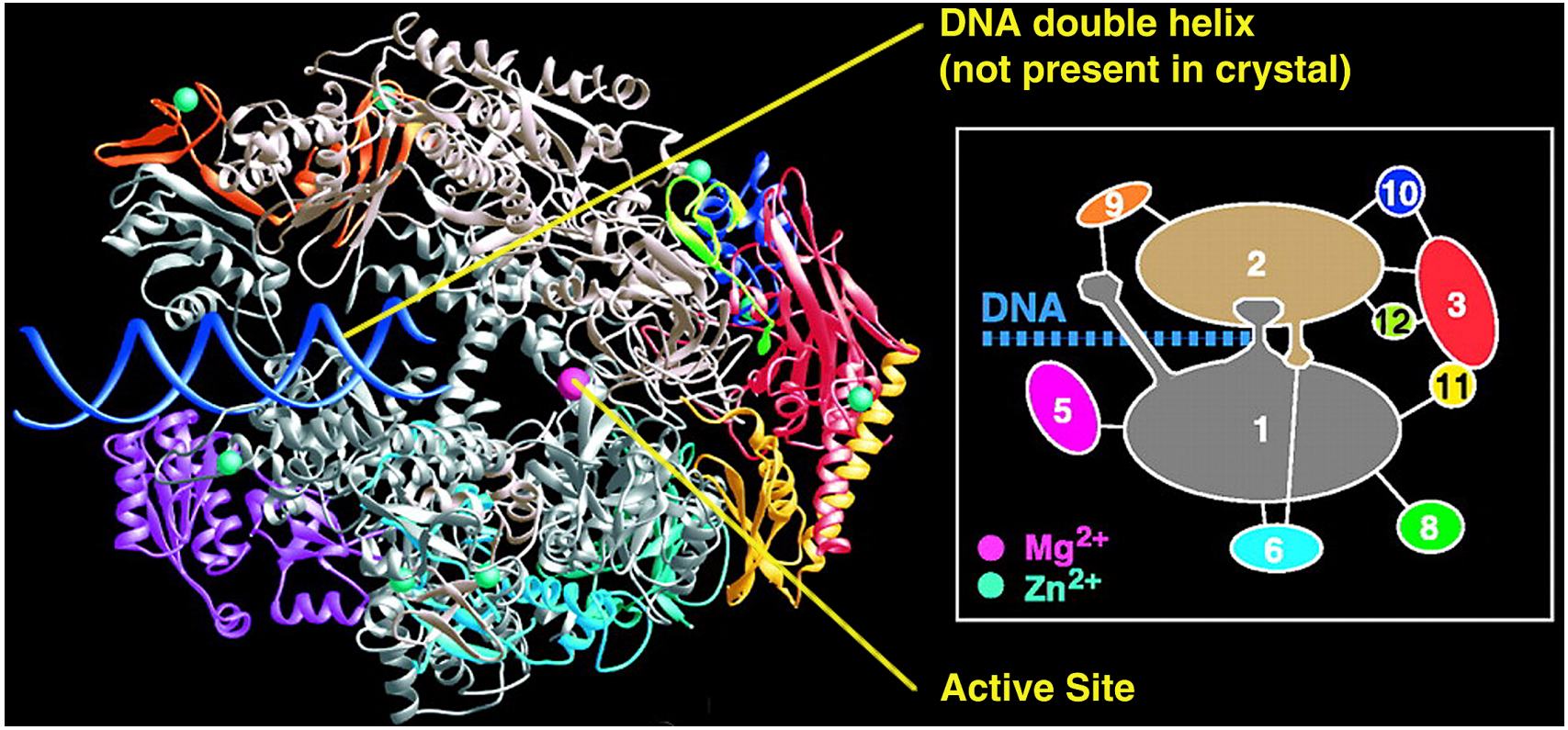

Ribbon diagram of RNA polymerase II backbone model. The inset shows a color-coded schematic of the 10 subunits and their interactions.

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